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OXYGEN UPTAKE AND TRANSPORT IN THE PROSOBRANCH MOLLUSC *BUSYCON CANALICULATUM* (L.) II. INFLUENCE OF ACCLIMATION SALINITY AND TEMPERATURE

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At summer temperatures and in high salinity waters, the prosobranch mollusc *Busycon canaliculatum* (L.) utilizes an unusually large fraction of the oxygen in its blood, permitting a relatively high rate of oxidative metabolism (Mangum and Polites, 1980). Like most molluscs, the conch regulates physical and chemical conditions in the blood either very weakly or not at all. Still, the species is found in both polyhaline and euhaline waters from Cape Cod to Florida, where it may experience an annual temperature change in excess of 20° C and a salinity range of 18 to 35‰. This magnitude of environmental change should seriously perturb the performance of the oxygen transport system. At the mean annual temperature in the middle of the geographic range (10° C), the oxygen affinity of conch hemocyanin is four times higher (P_{50} = 3 mm Hg at pH 7.9 and 35‰ salinity) than at 22° C (P_{50} = 12 mm Hg under the same conditions) (Mangum and Lykkeboe, 1979). At 18‰ salinity, the cooperativity of oxygen binding sharply decreases, which could seriously impair oxygen uptake by lowering the oxygenation state of the blood at branchial P_{O_2}.

In addition to direct effects on the oxygenation of the carrier molecule, temperature and salinity may influence the respiratory properties of the blood indirectly, by means of effects on blood pH. In many animals blood pH increases at low temperature or low salinity, and this change could be especially important in *B. canaliculatum* because pH is an unusually strong and complex determinant of hemocyanin oxygenation (Mangum and Lykkeboe, 1979). In crustaceans, the changes in blood pH accompanying adaptation to low salinity waters oppose the respiratory effects of salt loss from the blood. While the salt loss lowers oxygen affinity, the concomitant pH increase raises oxygen affinity, and the net result is an oxygen transport system that works better under the particular combinations of environmental and behavioral conditions that accompany the migration upstream (Mangum and Towle, 1977). The changes in blood pH are intimately related to the regulation of salt and water content of intracellular and extracellular body fluids. In gastropods, regulation of blood salts is known only at very low salinities not within the range tolerated by neptuneids (Staaland, 1970). While cell volume appears to be regulated quite strongly (Staaland, 1970), the magnitude of the changes in free amino acids is conspicuously variable among different species (Schoffeneils and Gilles, 1972), which implicates the participation of other osmolytes that may have quite different effects on blood pH. Therefore, the net result of low salinity adaptation on the oxygen transport system cannot be predicted.

We have studied the effects of temperature and salinity an oxygen transport, with emphasis on the relationship to total oxygen uptake.

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Methods and Materials

The measurements were made on animals collected throughout the year to minimize the difference between natural and experimental conditions. The animals were held at a particular combination of temperature and salinity for a minimum of 1 week, if the environmental change was small, and a maximum of 3 weeks.

The procedures used in collecting the respiratory data are described by Mangum and Polites (1980). Osmolality was measured with an Osmette freezing point osmometer, chloride with a Buchler-Cotlove chloridometer, ammonia by the phenol hypochlorite method (Solòrzano, 1969; Gravitz and Kleye, 1975) and total ninhydrin positive substances as described by Henry and Mangum (1980). The free amino acid composition of pedal muscle tissue was determined with a Technicron Auto amino acid analyzer, as described by DuPaul and Webb (1970). The tissue was excised, blotted dry, extracted with 80% ETOH for 2 days, and then dried at 60° C to constant weight.

After the profound disturbance accompanying changes of the medium or transfer to another vessel, conchs often take a very long time to emerge from their shells and resume the exchange of metabolites with the medium. Therefore, the measurements of ammonia and TPNS excretion were made in the running sea water systems of the laboratories of the Virginia Institute of Marine Science. Nitrogen excretion was first determined at high salinity (33‰) at the Wachapreague Laboratory by placing animals (starved for 1 week) in a 10-liter container of running sea water. After ascertaining that the spihon was extended, the tap was closed and the addition of nitrogen compounds to the medium was assayed for 1 hr. The animal was then transported to the Gloucester Point Laboratory, allowed to acclimate for 7 days to running 18‰ water, and the experiment was repeated.

The behavior pattern also seemed to influence the oxygen uptake measurements, in that the period required for emergence after disturbance was highly individual. Paired observations on the same animals acclimated to different salinities were made of oxygen uptake as well; in this instance the low salinity animals were held in very large (60-liter) containers of standing, aerated water.

Data analyses were performed according to Student's t test, for either paired or unpaired observations.

Results

Behavior

The ambient temperature varied from 21 to 24° C during the experiments at high temperature. While the animals appeared to behave normally at temperatures lower than 23° C, they were conspicuously sluggish at 24° C, especially at low salinity, and considerable mortality occurred ($> 50\%$). The same pattern occurred in *B. carica*. This finding, although highly repeatable, seems somewhat anomalous in view of the geographic ranges of the two species, although local reports of "estivation" and offshore migration in mid-summer may explain the anomaly.

At 10° C locomotion in *B. canaliculatum* is reduced but not entirely absent, and the siphon is still extended, though less than at higher temperatures. Below that threshold, which delimits the abrupt transition between summer and winter conditions, conchs burrow fully into the sediment and retreat more than halfway

Table I

Effect of acclimation salinity and temperature on the osmotic and ionic properties of the blood in Busycon canaliculatum. *Mean ± s.e. (N).*

	6° C	10° C	22–24° C		
Osmolality milliosmoles					
Water	546	971	466		915
Blood	560 ± 1 (11)	977 ± 3 (7)	468 ± 1 (6)		918 ± 2 (11)
Chloride (mM)					
Water			271	535	554
Blood			257 ± 1 (8)	518 ± 2 (8)	529 ± 3 (9)
Blood pH (pedal)					
33–34‰	7.923 ± 0.024 (24)	7.945 ± 0.015 (38)	7.853 ± 0.009 (24)		
20‰	8.015 ± 0.039 (16)	8.007 ± 0.029 (7)	7.910 ± 0.023 (16)		
Pedal blood ammonia (μM)					
High salinity (31‰)	187 ± 14 (5)	203 ± 18 (18)	123 ± 16 (7)		
Low salinity (19‰)			129 ± (18)		
Branchial blood ammonia (μM), 20–33‰					
Postbranchial			34 ± 6 (11)		
Prebranchial			91 ± 10 (10)		

into their shells. Ventilation is virtually blocked and there is little evidence of locomotion. Local observers believe that the animals migrate offshore and "hibernate". While the movements of populations are poorly known, it is clear that the most active period of feeding and locomotion spans less than half of the year.

Blood osmotic and ionic composition

The blood of warm acclimated (22° C) animals tends to be slightly (but not significantly, $P > 0.05$) hyperosmotic to the medium throughout the range of salinity tolerance (Table I). The hypersomotic condition of the blood, which grows larger at low temperature, becomes highly significant at 6° C ($P < 0.001$). As shown earlier (Mangum and Lykkeboe, 1979), the blood at 22° C is consistently hypoionic to the medium with respect to NaCl and Mg^{+2}, isoionic in K^+, and hyperionic in Ca^{+2}, NH_4^+ and HCO_3^-, regardless of acclimation salinity. At low temperature, the blood-medium difference in inorganic osmolytes decreases (Mangum and Lykkeboe, 1979), which more than accounts for the changes in osmolality (Table I).

Pedal blood pH rises at low temperature, at least in the range 10 to 22° C (Table I), but the change is quite small ($\Delta pH/\Delta C = -0.008$ at both salinities). When the temperature of a blood sample held *in vitro* is changed anaerobically, the change remains smaller (-0.012) than that of pN, the neutral point of water, over the same temperature interval ($\Delta pN/\Delta C = -0.014$). No further increase in blood pH occurs *in vivo* at 6 to 10° C (Table I), which may be due to an accumulation of acidic metabolites in the absence of blood flow (see below).

Blood pH rises ($P < 0.05$) at low salinity (Table I), but this change is also much smaller than observed in other groups of euryhaline animals (Mangum, 1976).

Intracellular free amino acids and nitrogen excretion

The size of the total free amino acid pool in pedal muscle cells varies directly with acclimation salinity (Table II). However, the change is very small and the pattern differs from that in lamellibranchs, which has been investigated more intensively. Alanine drops sharply at low salinity, as in other gastropods (Schoffeneils and Gilles, 1972; Bedford, 1971) and ornithine decreases appreciably as well, neither of which could have been detected by the methods used in previous

TABLE II

The concentration (μM/g dry wt) and % composition of free amino acids in pedal muscle fibers of Busycon canaliculatum *acclimated to high and low salinity at 23 to 24° C.*

	18‰		33‰		Δμm/g
	μM/g	% total	μM/g	% total	
taurine	114.2	46.8	94.1	31.7	−20.1
aspartic acid	26.1	10.7	28.1	9.5	2.0
threonine	7.2	3.0	3.6	1.2	−3.6
serine	13.5	5.5	11.7	3.9	−1.8
glutamic acid	24.5	10.0	18.3	6.2	−6.0
proline	19.3	7.9	17.4	5.9	−1.9
glycine	3.1	1.3	10.4	3.5	7.3
alanine	15.0	6.2	57.6	19.4	42.6
valine	—	0	7.1	2.4	7.1
methionine	3.8	1.6	—	0	−3.8
isoleucine	1.3	0.5	4.9	1.7	3.6
leucine	1.8	0.7	10.5	3.5	8.7
tyrosine	—	0	4.8	1.6	4.8
phenylalanine	2.2	0.9	2.0	0.7	−0.2
ornithine	1.7	0.7	20.1	6.8	18.4
lysine	6.8	2.8	6.1	2.1	−0.7
histidine	3.6	1.5	—	0	−3.6
Total	243.9	100.1	296.7	100.1	52.8

investigations. These changes are offset in large part by an increase in taurine, which has been reported before but not in this magnitude (Schoffeneils and Gilles, 1972). The net free amino acid change of 53 μM/g dry wt is only 17% of that in the clam *Rangia cuneata,* for example, over a comparable salinity interval (Henry and Mangum, 1979). Assuming the same intracellular water content in the two species, the molal change in *B. canaliculatum* (164 mM/kg cell H_2O) is only half that in the related neptuneid *Buccinum undatum,* over the same salinity range (Staaland, 1970). In view of the small change in free amino acids, it is not surprising that the levels of ammonia in the blood (Table I) do not change significantly with acclimation salinity. Nor is there a significant rise in ammonia and free amino acid excretion (Table III) at low salinity ($P < 0.05$).

The magntude of nitrogen excretion is somewhat unexpected. The total in *B. canaliculatum,* a predator and carrion feeder, is an order of magnitude lower

TABLE III

Nitrogen excretion in Busycon canaliculatum *Mean ± s.e. (N).*

Experimental (=acclimation) conditions	Ammonia excretion [μM/(g dry wt·hr)]	Free amino acid excretion [μM leucine/(gm dry wt·hr)]
A. Paired observations on intact animals		
15° C, 18‰	0.54 ± 0.05 (7)	0.33 ± 0.50 (7)
15° C, 30‰	0.49 ± 0.06 (7)	0.36 ± 0.41 (7)
B. Pedal muscle tissue		
5° C, 31‰	0.62 ± 0.03 (8)	
25° C, 31‰	1.10 ± 0.15 (8)	

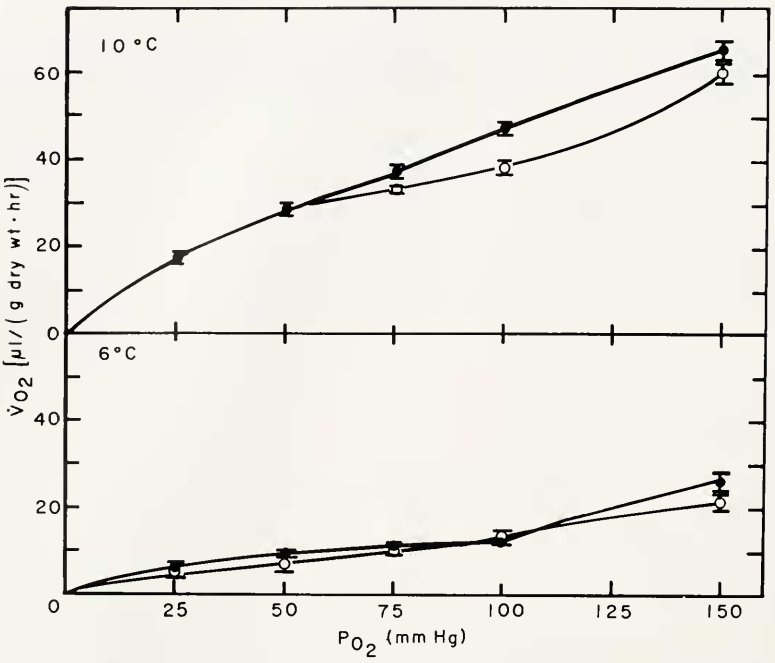

FIGURE 1. The influence of temperature and salinity on total oxygen uptake ($\dot{V}_{O_2}$) in *Busycon canaliculatum*. (Closed circles 31 to 34‰, (open circles) 18 to 19‰. Mean ± s.e. (N = 9).

at 15° C than in *R. cuneata,* an herbivorous filter feeder, at 22° C and the same acclimation salinity (18–22‰). This difference is unlikely to be due solely to the lower experimental temperature. At the slightly higher experimental temperature of 25° C, ammonia production of isolated muscle fibers taken from *B. canaliculatum* (Table III) is also less than in *R. cuneata* [3.6 µM/(g·hr) at 22° C; Henry and Mangum, 1980]. On the other hand, the magnitude of ammonia excretion in both animals and isolated muscle fibers is directly correlated with the rate of oxidative metabolism, which is considerably higher in *R. cuneata* (Henry and Mangum, 1980).

Oxygen uptake

The complex relationship between oxygen uptake and ambient P_{O_2} (Fig. 1 in Mangum and Polites, 1980) is not influenced by acclimation salinity, but the exponential change at high P_{O_2} becomes very small at low temperature (Fig. 1 in present report). Using paired observations on the same animals, the influence of a salinity change in either direction is clear. In 8 of the 9 individuals tested, oxygen uptake varies directly with acclimation salinity, and the mean decrease at low salinity, 22° C and ambient P_{O_2} 100 to 125 mm Hg is 26 (± 6) %. No paired observations were made at lower temperatures.

Oxygen uptake decreases sharply at low temperature. Using the data for both acclimation salinities, which do not differ significantly, the weight-corrected value for a 24.2-g animal at 100 to 125 mm Hg is 36 (± 3 s.e.) µl/(g dry wt·hr) (N = 28) at 10° C and 12.6 (± 2.5) µl/(g dry wt·hr) (N = 16) at 6° C. The

TABLE IV

Oxygen uptake ($\dot{V}_{O_2}$) of tissues isolated from Busycon canaliculatum. *18‰, 22° C. Mean ± s.e. (N).*

Tissue	$\dot{V}_{O_2}$ [μl/(g dry wt·hr)]	Difference from 35‰ (%)
Pedal muscle	87 ± 7 (29)	−22 ($P < 0.05$)
Cardiac muscle	484 ± 20 (5)	not significant
Pedal epidermis	235 ± 19 (5)	not significant
Gill	961 ± 26 (12)	not significant

temperature coefficients (Q_{10}) for total oxidative metabolism are 2.0 for the interval 10 to 22° C, and 14 for 6 to 10° C.

While the oxygen uptake of pedal muscle tissue taken from low salinity acclimated animals is significantly lower (Table IV) than that of high salinity-acclimated animals (Mangum and Polites, 1979), the rate in other isolated tissues does not change significantly ($P > 0.05$).

Ventilation

In warm-acclimated animals at 22° C, ventilation decreases by 17% to 13.4 ml/(g dry wt·hr) at 18‰ salinity, possibly due to a direct effect of low salt levels on the activity of the ciliary pump, as in Lamellibranchia (Ghiretti, 1966). At low temperatures, retraction into the shell results in a decrease of the cross-sectional area of the excurrent to a size that is too small for the probe to function without altering the flow properties of the stream, and the siphon will not extend far enough to make a tight connection with a manometer. Under these conditions no measurements were made.

Blood P_{O_2}, oxygen carrying capacity and oxygenation

Blood P_{O_2} does not change significantly with salinity, regardless of temperature (Table V), but it rises at 10° C. More notably, the relationship of blood P_{O_2}

TABLE V

Effect of acclimation salinity and temperature on the respiratory properties of the blood in Busycon canaliculatum. *Mean ± s.e. (N). Data for 31 to 34‰ and 22° C from Mangum and Polites (1979).*

	22° C		10° C	
	31–34‰	18‰	31–34‰	18‰
Postbranchial blood				
PO_2 (mm Hg)	25	25 ± 3 (6)	45 ± 6 (4)	48 ± 7 (3)
Hcy O_2 (%)	84	73	100	100
Prebranchial blood				
PO_2 (mm Hg)	2.3	2.1 ± 0.3 (5)	19 ± 3 (4)	19 ± 3 (5)
Hcy O_2 (%)	16	16	86	86
Pedal blood				
PO_2 (mm Hg)	5.5	7.1 ± 2.0	62 ± 8 (8)	
Hcy O_2 (%)	33	40	100	
Nephridial blood				
PO_2 (mm Hg)			12 ± 1 (3)	
Hcy O_2 (%)			82	
Postbranchial-prebranchial blood O_2 conc. (ml/100 ml)	2.25	1.89	0.78	

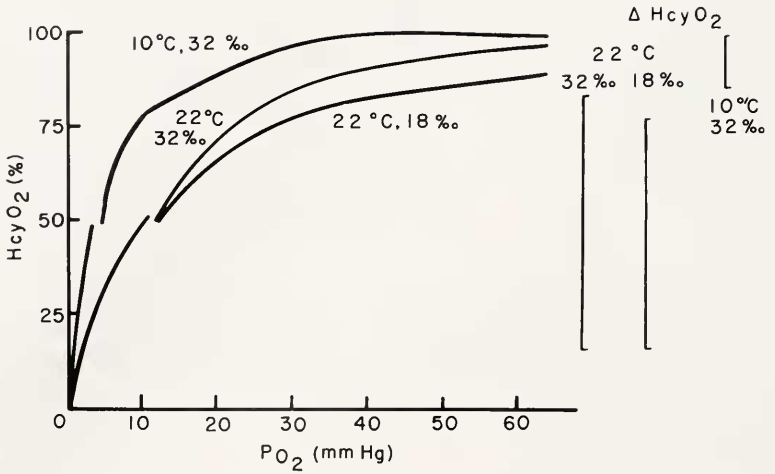

FIGURE 2. The influence of temperature and salinity on oxygen transport in the blood of *Busycon canaliculatum*. Vertical bars at right showing change in hemocyanin oxygenation. Data from Table V.

in different parts of the system shifts at low temperature. At 10° C, the margin of the partly retracted foot appears to be swollen with dark blue blood, which is more highly oxygenated than postbranchial blood (Table V). The hydrostatic pressure generated in the posture seems to distend the pedal epithelium, and it should reduce the diffusion distance between pedal blood and ambient water. Apparently the foot becomes a site of oxygen uptake.

If the volume of the blood increases at low salinity by about 15%, as in *Buccinum undatum* (Staaland; 1970), then oxygen carrying capacity should decrease measurably. In a total of 20 determinations on pedal blood and 26 on renal blood at various temperatures, there was no detectable effect of acclimation salinity. Although the copper content of blood diminishes in winter animals, presumably below 10° C (Betzer and Pilson, 1974), there is no evidence in our data of a significant decrease ($P < 0.50$) in the oxygen-carrying capacity of blood taken from animals acclimated to 10° C. In contrast to warm-acclimated animals (Mangum, 1979), there is no difference in absorbance at 345 nm between samples of pedal and renal blood ($N = 26$), suggesting little or no dilution of pedal blood, when the animals are retracted.

Using the data for oxygen equilibrium properties reported earlier (Mangum and Lykkeboe, 1979), the effect of low temperature and low salinity on the respiratory properties of the blood is summarized in Table V and Figure 2. The removal of divalent cations from the blood at 18‰ significantly reduces the cooperativity of oxygen binding to *B. canaliculatum* hemocyanin, which appreciably lowers its oxygenation state at a particular pH and P_{O_2}. The small increase in pH at low salinity does not significantly influence oxygenation, and there is no compensatory increase in P_{O_2} to enhance oxygenation. The net result is a decrease of about 16% in oxygen uptake by the blood. The oxygen uptake of many tissues is not directly influenced by acclimation salinity, but $\dot{V}_{O_2}$ of pedal muscle fibers decreases, which may account for the discrepancy between the estimated decrease of blood-supplied oxidative metabolism (16%) and the observed decrease of total oxidative metabolism (26%).

At 10° C the critical pH at which the reverse Bohr shift disappears increases to about 8.05, and thus the oxygen affinity of the blood increases more ($\Delta H = -17$ kcal/mole at pH 8.0) than it would if the Bohr shifts were not heterogeneous or if they were independent of temperature (Mangum and Lykkeboe, 1979). While the blood is more highly oxygenated at the gill at 10 than at 22° C, the venous reserve is also larger (Table V). Oxygen extraction from the blood decreases from 82% at 22° C to only 43% at 10° C. Using the erroneous assumption that oxygen uptake by the blood equals total oxygen uptake, the Fick estimate of cardiac output predicts an increase of 96 ml/(kg·min). A single successful measurement of heart rate, however, showed a substantial decrease (> 50%), which is unlikely to be reversed by a larger stroke volume. The discrepancy between the changes in heart rate and the Fick estimate of cardiac output suggest an increase in the relative importance of direct oxygen uptake by unvascularized tissue, which exaggerates the error in the Fick estimate. This suggestion is strongly supported by the data for blood P_{O_2}, which are distributed in the system differently at 10° C, and also by the change in total oxygen uptake (−56%), which agrees with that of heart rate.

At 6° C, we were unable to detect a heartbeat by visual observation of a large number of individuals, or by implanted electrodes (de Fur and Mangum, 1979). Although no measurements were attempted on the blood taken from sites such as the gill, now withdrawn deep into the shell, pedal blood P_{O_2} remained very high [70 (± 12 s.e.) mm Hg, N = 6].

Discussion

Blood pH in *Busycon canaliculatum* is relatively insensitive to environmental change, which precludes the possibility of restoring its respiratory function by opposing thermal or ionic effects with a concomitant alteration of acid-base status. Although the insensitivity is in part an intrinsic property of the blood, it is not easily explained by the ionization of structural groups on the hemocyanin molecule. If gross histidine content were the only factor determining the buffer properties of a protein-containing blood, the thermal behavior of conch blood would be the same as that of mammalian blood; both prosobranch hemocyanin and Hemoglobin A contain about 6% histidine (Van Holde and Van Bruggen, 1971). The departure cannot be attributed to the absence in conchs of other imidazole-containing proteins, which does not result in reduced thermal sensitivity of pH in many groups of animals (Mangum, 1973). In its thermal behavior *B. canaliculatum* blood, essentially a mixture of sea water and hemocyanin, resembles sea water ($\Delta pH/\Delta C$ of the CO_2 system = −0.007) more than a high imidazole-containing solution ($\Delta pH/\Delta C = -0.018$; Reeves, 1972). We suggest that the basis of the similarity is the unique character of *Busycon* blood, which *is* more sea water than protein in the quite literal sense (Mangum, 1979). At summer temperature the foot is apparently inflated with sea water, which appears to mix freely with blood, and it is blood pH at these temperatures which is unusual, not the values at 6 to 10° C. The direction of this departure, or an unusually high pH at 23° C, is the expected trend if the CO_2 system of sea water dominates the heterogeneous buffer system in the blood.

The relatively small increase in blood pH at low salinity may be due in large part to the very small change in intracellular free amino acids and concomitant alkalinization by ammonia. A similar correlation, a small change in blood pH with acclimation salinity in an animal that does not utilize free amino acids as

intracellular osmolytes, is found in the xiphosuran *Limulus polyphemus* (Mangum, Booth, deFur, Heckel, Henry, Oglesby and Polites, 1976). Unlike *Limulus*, the excretion of hydrogen ions *via* ammonia is very small in *Busycon*.

Alternatively, or perhaps in conjunction, it has been suggested that the component of the CO_2 system which is regulated homeostatically in decapod crustaceans is not H^+ but HCO_3^- (Truchot, 1978), which is believed to be actively excreted in exchange for Cl^-. Unlike these species, in which acd-base regulation has been studied in detail, *B. canaliculatum* is capable of only the most trivial ion regulation, and by no means is it clear that the process involves an active ion exchange. The machinery to maintain constant bicarbonate levels may simply be lacking in conchs. Our data on CO_2 levels in the blood are far too few to permit a meaningful conclusion, and it is clear that the subject of acid-base balance in osmoconformers is in need of serious investigation.

Regardless of its explanation, the conservatism of blood pH in conchs is not mysterious from an adaptive point of view. If the thermal response were determined by an imidazole alphastat, prebranchial blood pH at 23° C would be 7.7 and postbranchial pH 7.8, and the reverse Bohr shift would be a genuine physiological anomaly. The blood would return to the gill with fully 30% of its oxygen load, oxygenation at the gill would increase only to 89%, and oxygen uptake into the blood would be 12% less than it is. Similarly, a pH increase of 0.15 to 0.20 at low salinity, as in crabs, would not affect oxygen affinity, because it would occur in the pH range where the Bohr shift is virtually nonexistent (> 7.9). But a hypothetical increase in pH would elevate the carbonate content of the blood, enhancing the formation of Ca^{+2} and Mg^{+2} ion-pairs; cooperativity would probably disappear entirely and oxygen uptake at the gill would be further impaired. In fact, oxygen uptake is reduced at both low temperature and low salinity, and the change clearly results in part from unopposed effects of the environmental variables on the blood oxygen carrier. - We suggest that these effects are important in limiting the ecological range of the species, and in determining its behavior.

Our findings on the oxygenation state of postbranchial blood, and on the high mortality rate in the laboratory, suggest that 22 to 24° C is slightly above the optimum, which is supported by popular accounts of "estivation" or the disappearance of conchs in the heat of mid-summer. At a temperature only a few degrees lower (19° C), little mortality occurs in either running or recirculating water and a few observations suggest that total oxygen uptake may even increase possibly due to complete oxygenation of the carrier at the gill.

The oxygen transport system appears to be specilized for transitional and early summer temperatures. Below 10° C, corresponding to the period of about 6 months when conchs terminate active feeding and locomotion, the increase in oxygen affinity (to less than 2 mm Hg at physiological pH) effectively precludes the possibility of drawing on the oxygen supply in the blood. The temperature dependence of $\dot{V}_{O_2}$, as described by Q_{10}, abruptly rises. It is hardly surprising that the copper concentration of the blood falls, implying the suspension of hemocyanin synthesis during this period (Betzer and Pilson, 1974), which results in a drop in oxygen carrying capacity (calculated from Betzer and Pilson, 1974) to one-fourth of the peak value in the middle of the summer. Given the intrinsic physico-chemical sensitivity of conch hemocyanin and the limitations of the fluid convection systems, it is difficult to conceive of a compensatory physiological response that could offset the effects of the greatly increased oxygen affinity. We suggest that more active blood or water convection might entail little or no

net energetic advantage, and that the suspension of activity for half of the year is an adaptive response necessitated by the thermal sensitivity of the respiratory and metabolic systems.

The ecological limits of the oxygen transport system of *B. canaliculatum* are even more obvious at low salinity. As concluded earlier (Mangum and Lykkeboe, 1979), the intrinsic sensitivity of hemocyanin oxygen affinity to the loss of blood NaCl accompanying the invasion of estuarine waters appears to be offset by complex interactions of the molecule with the divalent cations and the anions of the CO_2 buffer system, resulting in little or no change. The response of P_{50}, however, is of little physiological importance in this species, in which the relevant oxygenation states are P_{85}, P_{15}, etc., or those at physiological P_{O_2} (see Table V). Above 40% these oxygenation states are strongly influenced by cooperative interactions of the oxygen-binding sites, which in turn depend on the divalent cations in the blood. Since the relationship is exponential in the physiological range, the small changes in divalent cations accompanying acclimation to low salinity can have large effects on aerobic respiration. If cooperativity were eliminated entirely while oxygen affinity remained unchanged, a very real possibility of further penetration into the estuary, oxygenation at postbranchial blood P_{O_2} would be only 64%, net oxygen uptake into the blood would drop to 1.60 ml/100 ml and the oxygen supply to the tissues would be half that observed (Table V, Fig. 2). Since the species does not actively regulate the osmotic concentration of its blood and since the mechanisms of reducing cell osmolytes is largely unknown, it is not obvious that the energetic demands of living in low and high salinity waters are very different. There is no reason to suppose, however, that the oxygen requirements compatible with life go down at low salinity. Although there are undoubtedly others, the determinants of salinity tolerance may include the properties of the oxygen transport system, which simply does not work as well in dilute waters.

Summary

1. The oxygen transport function of the blood in *Busycon canaliculatum* diminishes when either the ambient temperature or the ambient salinity is lowered.

2. At low temperature the oxygen affinity of the hemocyanin increases sharply, and blood P_{O_2} increases as well. Thus the blood cannot deoxygenate at the tissues.

3. At low salinity oxygen affinity does not change appreciably, but the loss of Ca^{+2} and Mg^{+2} from the blood reduces the cooperativity of hemocyanin-oxygen binding, requiring a higher P_{O_2} to achieve a given oxygenation state. Thus the blood does not oxygenate as highly at the gill, and total oxygen uptake decreases.

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